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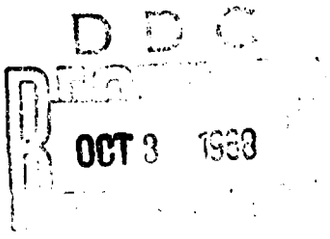

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EXPERIMENTAL INVESTIGATIONS ON THE DEVELOPMENT OF THREAD AND CHAIN FORMS IN BACTERIUM PRODIGIOSUM

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The literature on this subject contains numerous data on the changes in the form of the bacterial cell, both with respect to its size and with respect to the recurrence of individual cells or short and long chains (Minoux, Rolin, Stapp and Ruschmann, Peju and Rajat). However, so far there has been no systematic investigation of all of those factors which could be used in explaining the origin and development of these phenomena -- in other words, no such investigation has been performed on any species here. We would like to report here on several series of experiments which involve investigations of the thread and chain formation in Bacterium prodigiosum as a result of the addition of various neutral salts and which would appear to be of some interest in view of the problem of the effect of salt upon the individual bacterial cell, particularly also the problem of the way in which the growth and subdivision mechanisms are influenced. For purposes of comparison, we used several additional bacteria and proactinomycetes.

Under normal culture conditions (dextrose-probacite-agar), Bact. prodigiosum grows with highly mobile individual cells with a width of 0.5 mu and a length of 0.7 mu (Figure 1). Hereafter, the term "chains" will refer to the septed forms while the term "threads" will refer to the unsepted forms.

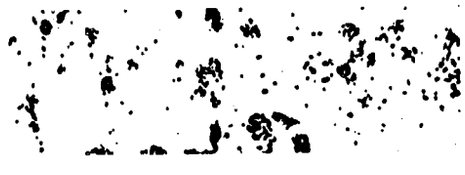

Figure 1. Bacterium prodigiosum. Standard culture. Enlarged 600 X.

Experimental Conditions

In the course of the investigations dextrose-probacite-agar (5 g dextrose, 6 g peptone, 4 g probacite supplied by the Maggi Company, 16 g agar, and 1,000 cm^3 of water) proved particularly good as culture substrate for Bact. prodigiosum. The various salts were added in a dissolved form. For this purpose we made three standard salt stock solutions and we then computed the concentration to be examined for 5 cm^3 of total liquid; we then placed the substance in the test tubes and we filled up with 2 cm^3 of distilled water. To these 2 cm^3 salt solution we added 3 cm^3 dextrose-agar 3/5; dextrose-agar 3/5, using the same nutrient volume, contains only 3/5 of the water volume given for standard agar. In this way we can work with a medium whose salt concentration can be adjusted as desired and whose nutrients and agar consistency remain unchanged. The nutrient solution used was unbuffered, except for those experiments which we will mention separately later on.

The various figures shown in the tables were obtained in the following manner: growth and pigment formation were determined macroscopically. The standard culture is brick-red. The values given in the tables for mucus formation refer to comparisons with control culture during our experimental phase. When the bacteria are over-inoculated, we can easily determine whether we are dealing with a non-slimy (-) slimy [mucous](+) or heavily slimy (+++) culture. The mobility was determined microscopically in water suspensions, in each case, in comparison to salt-free cultures.

Results of Experiments with Bact. prodigiosum

Alkali Salts

(a) Chlorides

The results of the experiments with alkali salts are shown in Tables 1-3. Figure 1 shows a picture of the normal culture. In detail, we have the following (Table 1):

Lithium chloride does not cause any actual thread or chain form as such. Up to a concentration of 0.19 mol/liter we find mobile cocci forms (0.5 mu). Cultures with additions of 0.20-0.48 mol/liter reveal a strong tendency toward the formation of double-cocci. At even higher concentrations, we can observe 4-member short cocci chains -- this is only a temporary phenomenon because these chains break up into individual cocci after 24 hours.

When we work with sodium and potassium chloride additions, the incubation time becomes considerably longer when we add more salt; for instance, when we put in 1.35 mol/liter, the incubation time is 7 days. Up to a salt addition of0.30-0.50 mol/liter, the cells are identical to the salt-free control cultures. In the range of 0.70-0.95 mol/liter, we can observe the formation of short-chains which soon fall apart again as the development process continues; from 1.00 mol/liter on up we observe long, unsepted threads. These are very slimy and no longer produce any pigment. The short chains are straight, long-drawn and narrow or they come in the form of angles whereas the threads are heavily wound.

Table 1. Effect of Alkali Chlorides on Bacterium prodigiosum

(a) Salz	LiCl		NaCl			KCl		
mol Liter	0,50	0,80	0,80	1,00	1,40	0,80	1,00	1,40
Wachstum (b)	+ + +	+ +	+ +	+ +	+	+ + +	+ +	+ -
Farbe (c)	rot (k)	rosa (l)	rosa (l)	weiß (m)	weiß (m)	rot (k)	rosa (l)	weiß (m)
Schleim (d)	+	+ +	+	+ +	+ +	-	+ -	+
Bakterienform und -länge (e)	Diplokokken 1 μ (f)	Kokk.-Kett. 2 μ (g)	Ketten 8 μ (h)	Kett. u. Fad. 10—20 μ (i)	Fäden 25 μ (j)	Ketten 5 μ (h)	Ketten 15 μ (h)	Fäden 30—50 μ (i)

(a) Salz	RbCl		CsCl			NH_4Cl		
mol Liter	0,80	1,00	0,03	0,07	0,10	0,80	1,00	1,10
Wachstum (b)	+ -	+ -	+ +	+ -	+ -	+ + +	+ +	+ -
Farbe (c)	weiß (m)	weiß (m)	rosa (l)	weiß (m)	weiß (m)	rosa (l)	rosa (l)	rosa (l)
Schleim (d)	+	+ +	+ -	+ +	+ + +	+	+	+ +
Bakterienform und -länge (e)	Fäden (j) 10 μ	Fäden (j) 50 μ	Fäden (j) 10 μ	Fäden (j) 40 μ	Fäden (j) 75—100 μ	Ketten (h) 10 μ	Ketten (h) 30 μ	Fäden (j) 80 μ

Key: a. Salt
b. Growth
c. Pigment
d. Mucous [slime]
e. Bacteria form and length
f. Diplococci
g. Cocci chains
h. Chains
i. Chains and threads
j. Threads
k. Red
l. Pink
m. White

For growth and slime formation (these symbols also apply to the following tables): +++ = heavy; ++ = good; + = moderate; + - = very little.

Table 2. Effect of Cesium and Ammonium Sulfate on Bact prodigiosum

mol/Liter	Wachstum (a)	Farbe (b)	Schleim (c)	1 Tag (d)	2 Tag (e)	3 Tag (f)
Cs_2SO_4						
0,009	+ +	rosa (g)	-	5 μ	1 μ	1 μ
0,010	+ +	rosa	+ -	25 μ	8 μ	1 μ
0,020	+ +	weiß (h)	+	45 μ	20 μ	8 μ
0,030	+	weiß	+ +	75 μ	75 μ	75 μ
0,040	+ -	weiß	+ + +	50 -200 μ	50—200 μ	50—200 μ
0,045	+ -	weiß	+ + +	50—200 μ	50—200 μ	50—200 μ
$(NH_4)_2SO_4$						
0,40	+ +	rosa (g)		3—5 μ	1 μ	1 μ
0,50	+ -	rosa	+	3—5 μ	5 -10 μ	10 μ
0,60	+ -	rosa	+	—	30 μ	30 μ
0,65	+ -	rosa	+	—	—	30 μ

Key: a. Growth
b. Pigment
c. Slime
d. 1st day
e. 2nd day
f. 3rd day
g. Pink
h. White

Table 3. Effect of Alkali Nitrates on Bact. prodigiosum

	$LiNO_3$	$NaNO_3$	KNO_3	$RbNO_3$	$CsNO_3$	NH_4NO_3
Mol/Liter	0,40	0,35 - 0,4	0,35 - 0,4	0,40	0,03 - 0,08	0,35 - 0,40
Wachstum (a)	+	++	++	++	+-	+++
Farbe (b)	rot (i)	rot (i)	rot (i)	weiß (j)	weiß (j)	rot-rosa (k)
Schleim (c)	—	+	+	+	++	+-
Bakterienform u. -länge (d)	Diplokokken Kokkenketten 2 μ (e)	Ketten Fäden 25 - 50 μ (f)	Ketten Fäden 25—40 μ (f)	Fäden 40 μ (g)	Fäden 100 μ (g)	Ketten 25 μ (h)

Key:
a. growth
b. pigment
c. slime
d. bacteria shape and length
e. diplococci, cocci chains
f. chains, threads
g. threads
h. chains
i. red
j. white
k. red-pink

The rubidium cultures reveal chains with an extraordinarily sharp arrangement or organization; although they are quite long (50 mu), the threads are quite straight.

In the cesium cultures, the straight, unpigmented threads remind us very much of the cultures with rubidium-chloride addition but they are even longer (75 mu) and they are formed even at very low concentrations (see Figure 2, for 0.04 mol/liter Cs_2SO_4). A major reduction in the entire vitality phenomena characterizes the toxic character of the salt.

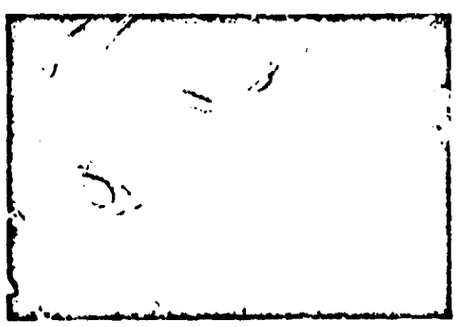

Figure 2. Bacterium prodigiosum, 0.04 mol/liter Cs_2SO_4 addition (dark-field coating). Enlarged 1,000 X.

When we add ammonium chloride, we get a chain form which is typical for ammonium salts, with very sharp septing; this form is heavily emphasized here. The pigment formation is not completely discontinued even at the still tolerable salt limit [boundary] concentrations and the cultures remain transparent and have a rather faded pink color.

Table 1 shows us that all alkali chlorides had a specific effect. But the chloride compounds of the particular elements reveal some common properties. All alkali-chlorides must be added in a minimum concentration of 0.80 mol/liter in order to produce the formation of a chain; only in the

case of CsCl is it very much lower, that is, 0.03 mol/liter.

The salt group reveals a quite uniform chain and thread length for the elements Na, K, Rb and NH_4 and for NH_4, that is, 25-40 mu, while the addition of LiCl gives us extremely low values(5 mu) and CsCl addition gives us extremely high values (75-100 mu). In this connection we might note that the element lithium, which is a peak element in the first group in the periodic system, approaches the magnesium of the second group in terms of its properties; this magnesium forms exclusively diplococci even at higher concentrations.

(b) Sulfates

High molarities of SO_4 ions (Table 2) are tolerated better than those of Cl-ions. Lithium, sodium, potassium, and rubidium sulfates do not have any effect on chain formation in Bact. prodigiosum. Only when we add cesium sulfate do we get uncommonly long cell threads (100-200 mu; see Figure 2, for 0.04 mol/liter Cs_2SO_4). The threads are preserved until the culture dies out. When we add ammonium sulfate, the microscopic picture is almost identical to that of the ammonium chloride cultures. The chains are sharply septed and the individual members are almost cylindrical.

(c) Nitrates

A comparison of the nitrate values in Table 3 with the corresponding chloride values (Table 1) shows us that chain formation can be caused by adding nitrate to exactly half of the salt volume required for the chlorides. In addition, the specific effect of the cations is retained.

Earth Alkali Salts

From Table 4 we can easily see that the action mechanism of the earth alkali salts must be entirely different from that of the alkali chlorides.

Table 4. Effect of Earth Alkali Chlorides on Bact. prodigiosum

mol/Liter	Wachstum (a)	Farbe (b)	1. Tag (c)	3. Tag (d)	5. Tag (e)
$MgCl_2$					
0,1	+++	rot (i)	Kokken (f)	Kokken (f)	Kokken (f)
0,2	+++	rot	Kokken	Kokken	Kokken
$CaCl_2$					
0,2	++	rosa (j)	Kokken	Kokken	Kokken
0,3	+ -	rosa	Kurzketten	Kokken	Kokken
0,4	+ -	rosa	Kurzketten (g)	Kokken	Kokken
$SrCl_2$					
0,1	++	rot	Kokken	Kokken	Kokken
0,2	+ -	rosa	Kokkenketten (h)	Kokken	Kokken
$BaCl_2$					
0,1	+	rosa	Kokkenketten	Kokken	Kokken
0,15	-				

Key: a. Growth
b. Color
c. 1st day
d. 3rd day
e. 5th day
f. cocci
g. short chains
h. cocci chains
i. red
j. pink

Longer thread or chain formations were never observed. The short chains, whose individual links or members inclined toward the cocci form, are highly unstable and remind us of cultures with lithium addition. The level of the salt concentration required for chain formation decreases as the atomic weight increases in the sequence of the periodic system: calcium 0.30, strontium 0.20, barium 0.10 mol/liter. Magnesium additions did not lead to thread formation.

Effect of Acids and Bases

The addition of inorganic acids and bases does not produce any thread or chain formation in Bact. prodigiosum. Experiments were conducted with chemically pure hydrochloric acid, nitric acid, and sulfuric acid, with sodium hydroxide, potassium hydroxide, and barium hydroxide. On the other hand, the very smallest additions of boric acid produce sharply outlined chain forms (see: "The Promotion of Pollen Tube Growth Through Boric Acid" according to Th. Schmucker).

Similarly, all investigations with numerous organic acids and their salts, featuring a wide variety of chemical properties, came out negative as regards chain formation. Only the sodium and potassium salts of dichlorobenzol sulfonic acid cause chain formation in small doses; this can be explained on the basis of the dissociation here which is stronger when compared to the other organic acids used.

Salt Mixtures

In further experiments, an attempt was made to determine whether the chain formation capacity of the individual salts can be increased or eliminated entirely by combining identical cations with different anions, identical anions with different cations and different anions with different cations. These salt combinations can act either additively, that is to say, the chain-promoting, respectively, chain-inhibiting effect of both salt partners [components] is added up or accumulated, or they can reveal antagonistic effects. The ion antagonism in chain formation reveals extensive agreement with the phenomena which we know from the antagonistically influenced swelling and de-swelling experiments.

When two alkali chlorides are combined, the effects of the two individual salt partners [components] upon chain formation are largely cancelled out. On the other hand, if we combine NaCl or KCl with LiCl, then we get -- if we have high NaCl and low LiCl concentrations -- threads of greater length, that is to say, threads that are longer than those which we get if each individual partner [component] comes in the same concentration. An antagonism between Na or K and NH_4 exists to a small degree; in this connection we must mention that K and Na are by far less toxic than NH_4. The effect of the cation NH_4 will therefore prevail.

Salt mixtures of alkali chlorides with alkali sulfates or nitrates, and mixtures of alkali nitrates or also earth alkali chlorides, among each other, do not have an antagonistic effect. Instead, they have a favorable effect upon the length of the chain (additive effect).

On the other hand, again, combinations of alkali and earth-alkali salts or of earth alkali nitrates among each other do act antagonistically; these are observations which were made long ago in connection with physiological studies. Exceptions do exist with respect to ammonium and lithium compounds [combinations]; here it is first of all the ammonium properties which dominate whereas, when we add lithium to earth alkali chlorides, the de-swelling cation effects of both partners (the cocci chains, in each case) are completely cancelled out in certain mixture proportions — yes, we might indeed even find that the plasma is influenced in the opposite direction (chains of little rods).

Figures 3 and 4 illustrate a few examples of the ion-additive effect when we use salt mixtures.

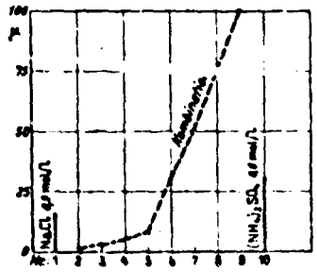

Figure 3. Effect of a mixture of sodium chloride and ammonium sulfate on Bact. prodigiosum. The ordinate at 1 represents the length of the thread for 0.90 mol/lit NaCl addition; the ordinate at 10 shows the thread length for 0.60 mol/lit $(NH_4)SO_4$. Points 2-9 show the length for various mixture proportions involving the 2 combination partners.

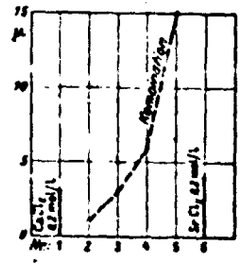

Figure 4. Effect of a mixture of calcium and strontium chloride on Bact. prodigiosum. For explanation, see Figure 3.

As far as the antagonistic effect of salt mixtures is concerned, we are dealing here with the following: the chain-forming factors of both salts are severely inhibited or completely cancelled out through this cooperation. We find a very strong ion antagonism in the combination of NaCl and CsCl, as we can see from Table 5. Thread formation can no longer be observed when we have a cesium-chloride concentration of as little as only 0.01 mol/liter.

Table 5. Antagonistic Effect of the Salt Combination NaCl-CsCl Upon Thread Formation in Bact. prodigiosum

	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ
	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ	1 μ

The following compilation shows us under what combinations of salts we get an additive or an antagonistic effect:

(a) Additive effect:
alkali chloride + alkali nitrate
alkali chloride + alkali sulfate
alkali nitrate + alkali nitrate

(b) Antagonistic effect:
alkali chloride + alkali chloride
alkali chloride + earth alkali chloride
earth alkali chloride + earth alkali chloride
alkali salts + lithium salts
alkali salts + salts of organic acids

Quantitative and Qualitative Changes in Standard Medium When Salt Is Added

In order to be able to determine whether a certain quantity of carbon and nitrogen will promote or reduce any possible chain formation, we varied the percentage content of the standard medium in terms of peptone, dextrose, and probacite. Without the addition of effective salts, there is no change in the morphological form, except in the case of the typical nutrient deficiency cells (involution forms in case of poor growth), when the minimum measure of carbon, which is 0.10%, and nitrogen, which is 0.005%, is not reached.

In salt media, on the other hand, a certain ratio between carbon and nitrogen is decisive for the length of the chains and threads. When the maximum value for the carbon source is exceeded, respectively, when the minimum value for the nigrogen source is not reached, the chain length is again reduced. The optimum values for the content in terms of dextrose and peptone, when combined, give us the best chain lengths. For prodigiosum cultures, the following are best: dextrose = 2%, peptone = 0.01%.

Table 6. Optimum Values for Dextrose and Peptone for Chain Length in Bact. prodigiosum

Dextrose Gew. % (a)	Peptone Gew. % (b)	Fadenlänge bei Zusatz von 1,0 mol/Liter $(NH_4)_2SO_4$ (c)
2,00	0,01	100 µ
2,00	0,005	75 µ
1,00	0,01	25 µ
0,10	0,40	10 µ
0,01	1,00	2--4 µ

Key: a. Dextrose in % by weight
b. Peptone in % by weight
c. Thread length when we add 1.0 mol/liter $(NH_4)_2SO_4$

When we have a favorable carbon:nitrogen variation, we can achieve an increase in the length of the chains by 5-7 times that of the control cultures. These results bring up the following question: might not salt concentrations smaller than the customary concentrations suffice for chain formation when we have a favorable carbon-nitrogen value ratio? But all of the experiments concerned with this came out negative. From this we can conclude the following: a minimum volume of salt must be added to the nutrient medium in order to get any chain formation at all. On the other hand, the chain length can be increased many times by an optimum carbon/nitrogen ratio. In connection with the qualitative change in the nutrient substrate the dextrose-carbon source was replaced with cane sugar, ammonium sulfate, glycerin, soluble starch, dextrin, pectin, and mucin; the peptone-nitrogen source was replaced with asparagin, potassium nitrate, ammonium sulfate, and alanin. As the chain-forming salt, we added a mixture of $NaNO_3$ 0.40 mol/lit and Na_2SO_4 0.20 mol/liter.

When we use cane sugar, ammonium tartrate and potassium nitrate, ammonium sulfate, and alanin, the experimental results are the same as when we add the right amount of dextrose and peptone. If we use glycerin as carbon source, then the length of the chains formed is reduced considerably (maximum values 5 mu as against 25 mu in dextrose control).

The polysaccharides starch, dextrin, pectin, and mucin completely suppress chain formation also in salt solutions; when the cultures grow nicely, we get cocci forms whose longitudinal dimensions are below those of the standard cultures, as we can see from Figures 5a and 5b.

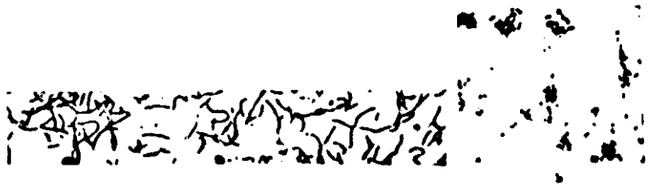

Figure 5. Bact. prodigiosum; enlarged [illegible] times. (a) 2% dextrose, 0.4 mol/liter $NaNo_3$ + 0.2 mol/liter Na_2SO_4. (b) 2% starch, 0.4 mol/liter $NaNO_3$ + 0.2 mol/liter Na_2SO_4.

There are two possibilities which we might mention in order to explain this:

1. In the case of Bact. prodigiosum, chain formation depends on specific carbohydrates (dextrose, cane sugar, ammonium tartrate).

2. The polysaccharides are not utilized as efficiently as the above-mentioned carbohydrates. If starch is the carbon source, then the starch reaction is still definitely positive after 6-10 days of cultivation; in other words, the ratio between carbon and nitrogen which can be used by the organism is lower than the ratio computed; there can then be no chain formation.

Physical Change in Salt Nutrient Media

The form of the little rods cannot be converted into the thread form by means of a pure physical change in the standard medium. In salt media, on the other hand, the length of the threads can be influenced by a number of physical factors.

For instance, when the pH value was varied, it was found that the chain formation was severely inhibited in acid and alkaline media, respectively, that it was entirely cancelled out, and that the optimum chain length is found in the neutral range [region].

In the salt media, the pH value was adjusted to pH = 4-6 (citric acid/hydrochloric acid), by means of buffer solutions, as well as to a pH = 6-7.5 (phosphate buffer), pH = 7.5-9 (citric acid/soda lye). Since the ions in the buffer solution, for their part, can again antagonistically influence the chain formation, glycocol mixtures were also used here, in addition, for buffering.

Table 7. Dependence of Chain Formation in Bact. prodigiosum on the pH Value When Ammonium Sulfate Is Added

$(NH_4)_2SO_4$ mol/Liter	pH = 5,6	pH = 6,8	pH = 8,4	unbuffered (a)
0,4	2 μ	2 μ	3 μ	2 μ
0,5	2 μ	12 μ	2 μ	20 μ
0,6	2 μ	12 μ	kein Wachstum (b)	25 μ
0,7	2 μ	25 μ	kein Wachstum	70 μ

Key: a. unbuffered
b. no growth

The variation of the agar consistency, in the presence of salt, gives us a longer, more lasting chain form on 2% of agar. Due to the increased agar swelling, the water, necessary for the complete division of the chain links, is bound too firmly to the substrate.

When we change the temperature, the surface tension, and the relative humidity in the air, we were unable to observe any differences with respect to the length of the chain.

Comparative Salt Experiments With Some Additional Microorganisms

The preceding salt experiments with Bact. prodigiosum showed that this organism does not react uniformly with different salts and that even chemically closely related substances produce entirely differently shaped forms. Investigations were now performed with a series of additional organisms in order to determine whether certain agreements exist in their behavior toward salts. The following were investigated as comparative organisms: Bact. coli, Pseudomonas fluorescens, Bac. subtilis, Bac. mesentericus, Bac. mycoides (as the typical chain formers) and some proactinomycetes from the "ruber" group (because of the fact that the mycelium normally decomposes very soon).

In summary, we can report that Bact. coli grows very poorly on salt nutrient media, that longitudinal little rods are formed in individual cases, and that we get short chains very rarely (RbCl 0.5-0.7 mol/liter 7 mu, NH_4Cl 0.4 mol/liter 4 mu, CsCl 0.05-0.07 mol/liter 5 mu).

Bac. fluorescens behaves in a manner similar to Bact. prodigiosum and forms threads and chains when we work with roughly the same salt concentrations.

Bac. mesentericus is somewhat more resistant against the effect of salt but it may produce chain forms in certain cases (NaCl 1.20 mol/liter 25 mu, CsCl 0.09 mol/liter 25 mu, NH_4Cl 0.60 mol/liter 25 mu). Its reaction to lithium salt is interesting; in contrast to all of the other observations with the other bacteria investigated, we get longer threads here (30 mu). It is to be assumed that the colloid-chemical composition of the plasma albumen of Bac. mesentericus is different from that of, for instance, Bac. prodigiosum.

In Bac. subtilis, it is only the spore formation which is delayed or completely inhibited when we add salt; the original chain form is retained and the longitudinal dimensions of the individual links are not altered.

Bac. mycoides and the proactinomycete strains likewise are extraordinarily ressistant against salt addition. Considerably larger salt quantities are tolerated here (for instance, in the case of Bac. mycoides: 2.10 mol/liter NaCl, 2.50 mol/liter KCl, 1.40 mol/liter NH_4Cl, a saturated K_2SO_4 solution); there is no standstill in the growth, nor is there any change in the cells. Involution forms were observed only in LiCl and $MgCl_2$. For the proactinomycetes, the boundary concentrations have the following values: LiCl = 0.8 mol/liter, NaCl = 1.6 mol/liter, KCl = 1.8 mol/lit, RbCl = 0.7 mol/liter, CsCl = 0.01 mol/liter, NH_4Cl = 1.2 mol/liter. When these concentrations are exceeded, the growth stops but there are no morphological changes before that.

In particular, we never get threads in Bac. mycoides; instead we always only get chains, in other words, also when we add cesium, whereby Bact. prodigiosum forms threads without lateral walls.

Investigations were performed on Bac. mycoides in order to get chain-free cultures. The fact that this is possible, at least, emerges from the work by Stapp and Zycha (1931), Molzmuller (1909), as well as Roelcke and Intlekofer (1938) (on Bac. anthracis); these authors describe the transformation of the R-form with the long cell chains into the S-form with the shorter cell forms.

The gradual transformation of the chain form into the little rod form was achieved successfully as a result of the regular over-inoculation of at least 3-week old cultures (in other words, not as a result of the over-inoculation of young cultures) on substrates with a high carbon content (2% dextrose) and a high nitrogen content (0.60% peptone). The rod form (S-form) then has lost its capability of growing in the form of the spiral windings which are so characteristic for Bac. mycoides and which are very easily visible with the naked eye; on the other hand, the mycoides cultures, on nitrogen-poor nutrient medium with 0.10% peptone, continue the spiral windings. The rod form can again be converted into the chain form if we re-inoculate on potato agar (0.06% nitrogen) or nitrogen-poor dextrose agar.

Discussion of Results

In the alkali salts, the influence of the anions asserts itself still rather strongly whereas in the earth alkali salts the cation properties prevail.

The salt concentrations necessary for a chain or thread formation in Bact. prodigiosum or, speaking in terms of colloid chemistry, the increasing de-swelling capability of the salts increases in accordance with the anion series:

$NO_3 <$ (J, Br) $<$ Cl $<$ Benzoate $<$ Formiate $<$ Tartrate $<$ $SO_4 <$ Citrate

swelling ions (chain-threads) — de-swelling ions (cocci)

increased de-swelling $\longrightarrow$

respectively, in accordance with the cation series:

$Cs < Rb < NH_4 < Na < K <$ Li $<$ (Mg, Ca, Sr, Ba)

swelling ions (chains) — de-swelling ions (cocci)

increased de-swelling $\longrightarrow$

In connection with these ion series we might note that the position of the acid radicals of the organic acids and of the SO_4 ion and in the cation series, the sequence of the earth alkalies, refer to the concentrations at which a de-swelling (cocci form) becomes microscopically visible. (In the swelling ions, we can also take into consideration here the chain length.) This means the following: for citrate and barium, the salt concentrations, which lead to de-swelling, are the lowest; they are the most strongly de-swelling ions. Conversely, NO_3 and Cs swell most strongly and the salt volumes necessary here are the smallest.

These ion series, which have been set up for our experiments here, of course do not agree fully, but only in broad outline with the anion and cation series, such as Hofmeister established them for the swelling and de-swelling effect on albumin bodies.

Hofmeister's anion series:

$SCN < J < NO_3 < Br < Cl <$ Acetate $<$ Tartrate $<$ Citrate $<$ SO_4

increasing de-swelling $\longrightarrow$

Hofmeister's cation series:

$NH_4 < K < Na < Li < Mg < Ca < Sr < Ba$

increased de-swelling $\longrightarrow$

As the swelling increases, there is an increase in the total vitality inhibition; this is recognizable on the outside on the basis of the end of the partition (+ thread formation), increased slime and reduced pigment formation, as well as decreasing motion intensity. The plasma swelling accordingly is influenced to differing degrees by the ions; the permeability follows these changes in a specific manner.

The resistance of the bacterium cell toward sulfate additions can probably be explained on the basis of the property of the SO_4 ions which can form a large hydratation envelope; because of this, other dissolved substances, for instance, sugar, are intensively displaced from the solution and into the cell, that is to say, an addition of SO_4 can lead to an

increase in the metabolism. As a matter of fact, in cultures with the maximum possible sulfate concentrations, we can still observe abundant growth which is at least the same as that of the control cultures, if not more. An exception here involves cesium- and ammonium-sulfate. In addition, the SO_4 ion, because of its de-swelling capacity, reduces the permeability, so that there is in practice hardly any major penetration of the salt and so that we can therefore have a disturbing influence on the growth and partition mechanism (cf, Walter).

To explain the chain formation in cesium- and ammonium-sulfate media, we might once again point out the extraordinarily high toxicity of both cations which perhaps work against the abovementioned properties of the sulfate ion. When we use lithium sulfate, the de-swelling properties of the anion and the cation are added together, whereby the cation has the greater toxicity.

In the preceding discussions of our experiments, we already mentioned the fact that bacteria chains break down into their individual links only after a few days of cultivation, whereas the threads retain their form. This phenomenon can perhaps be interpreted as follows:

When we add salt, the partition mechanism is slowed down in a primary phase due to processes influenced by colloid chemistry, whereas the longitudinal growth process continues uninhibited. As a result of this there is no final separation of the individual units which develop during partition and we get chains whose members consist of little rods (1-2 mu long, 0.5 mu wide). During the following secondary phase, longitudinal growth is inhibited while partition is continued to the end, although at a slower pace. As the result of the formation of a separating wall, the chains then fall apart into their individual members which however differ from the control cultures without the salt addition by virtue of their greater length. The secondary phase is reached after 2 or at most 3 days of cultivation -- unless this is preceded by a longer period of incubation (NaCl, KCl).

(The following remarks were added during the correction of the proofs: C.N. Hinshelwood [The Chemical Kinetics of the Bacterial Cell, Oxford, 1947] established a theory for the occurrence of chains in Bact. lactis aerogenes and their subsequent decomposition; according to this theory the division, only, is inhibited under certain conditions, whereas longitudinal growth is not influenced at all. In the course of bacteria development, the factor [an enzyme system] responsible for the partition becomes accustomed to the rather unusual medium and the balance between partition and growth is restored and the result is of course the breakdown of the chain.

But this assumption can be applied to Bact. prodigiosum only to a limited extent because a gradual adaptation of the cultures through over-inoculation onto salt nutrient media could not be observed. Hinshelwood describes only a few such experiments. This is why the concept of "adaptation" does not seem to be quite justified in the sense of a revival of the partition factor; otherwise the same cultures would either have to grow

normally in case of over-inoculation on salt nutrient media or they would have to grow at least with reduced incubation or accelerated chain decomposition.)

When we use very high salt concentrations, we get a thread formation. In these cases, the partition mechanism seems to be compressed completely, while growth in terms of longitude is more intensive than in the normal cultures. Later on, the growth slows down although there are still no partition phenomena here. Accordingly, the threads cannot fall apart into the individual component parts; instead, they are preserved in this form until they die off; partition is definitely and finally inhibited.

Here we might also point out the following very important facts. A salt can act in a specific concentration upon the formation of the chain shape only if it is added within the logarithmic phase of bacteria development, that is to say, during the first 8 hours. Any subsequent additions of salt no longer change the shape of the organisms, even though the bacteria development may not yet be complete. In this connection we also have a gradual transition from the thread form via the short-chain form to the long rod form. Looking at the example of cesium sulfate (0.04 mol/liter), this means that we get long threads when we add salt up to 3 hours after inoculation, that we get short chains up to 6 hours after inoculation, and finally only long rods (Table 8).

Table 8. Time Dependence of Salt Addition (0.04 mol/l Cs_2SO_4) After Inoculation in Bact prodigiosum

(a) Salzzugabe	(b) Mikroskopisches Bild am 2. Kulturtag: B. [illegible]	(c) Länge	(d) Wachstum
vor der Beimpfung (e)	Ketten (g)	30—75 µ	+ –
½ Std nach Beimpfung (f)	Fäden (h)	30—75 µ	+ –
1 „ „ „	Fäden	30—75 µ	+ –
2 „ „ „	Fäden	15—50 µ	+ –
4 „ „ „	Kurzketten (i)	19 µ	+
6 „ „ „	Kurzketten	2–7 µ	+
8 „ „ „	Kurzketten	2—7 µ	+
12 „ „ „	Langstäbchen (j)	2 µ	++
24 „ „ „	Langstäbchen	2 µ	++

Key: a. Salt addition
b. Microscopic picture on second day of cultivation
c. Length
d. Growth
e. Prior to inoculation
f. 1/2 hour after inoculation
g. Chains
h. Threads
i. Short chains
j. Long rods

There are tw possibilities for explaining this fact: (1) the Bacterium cell is so heavily taxed during the first hours of development as a result of metabolism and partition that it is not at all capable of performing any additional defensive measures against the action of the salt (perhaps through the formation or activation of a substance which would work against chain formation); (2) The chain formation depends on the pH; as we said before, it is inhibited by acid reaction.

In the first case, talking in terms of old standard cultures, the assumed [introduced] anti-chain substance would have to be enriched (as v. Denffer was able to establish an inhibition substance in aging diatomea cultures) and it would have to be possible to add isolated, young salt cultures. Theoretically, there would not be any change in the salt cultures. Conversely, talking in terms of salt cultures, a typical, chain-formation promoting substance would have to be developed and enriched and a concentrate of bacteria-free (sterile filtration through membrane filters) older salt solution would have to promote chain formation, when added to the standard cultures. Both of these possibilities were investigated. But the experiments came out negative.

One might furthermore assume that culture liquids of typical chain forming substances (the prototype used here was Bac. mycoides) contain a substance which promotes chain formation. However, neither in bacteria-free mycoides filtrate, nor in double cultures of Bac. mycoides and Bact. prodigiosum, separated by a dialysis membrane, was it possible to observe chain growth (or thread growth) in the latter [Bact. prodigiosum]; the cultures did not reveal any deviations from the standard culture.

The second assumption -- the assumption of a pH dependence of chain formation -- agreed more satisfactorily with the experimental results. The pH value of an unbuffered standard culture during the first 24 hours dropped from 7.2 down to 5.2. In this connection, it does not change during the first 7 hours and it then drops to its final value within 18 hours. But the thread length decreases considerably already after 2-7 hours, in other words, before we have a measurable pH drop in the culture solution. It is now very obvious to assume that a pH change occurs already after 2 hours following inoculation within the bacteria cell, a pH change which is caused or conditioned by the physiology of the metabolism here; this pH change influences the partition and growth process.

At this point we might refer to a bibliographic reference by Schardinger who describes the breakdown of a chain in a hydrochloric acid solution. We may assume with a great degree of probability that chain decomposition in prodigiosum cultures after 1-3 days of cultivation is based on the drop in the pH value. The moment of chain breakdown depends on the salt we use and its concentration because bacteria development and the pH drop are of course slower in salt media (Table 9).

Table 9. Dependence of Chain Breakdown on pH Value of Nutrient Solution

mol/Liter Salz-Zusatz (a)	(b) pH-Wert nach 12 Std	24 Std	36 Std	48 Std	Kettenzerfall nach (c)
Ca_3NO_4 0,010 . .	6,0	5,5	5,3	5,2	24 Std
Ca_3NO_4 0,020 . .	6,8	5,8	5,2	5,2	36 Std
KNO_3 0,3 . . .	5,8	5,8	5,8	5,8	7 Tagen (pH = 5,2)
$NaNO_3$ 0,3 . .	6,9	6,5	6,3	6,1	7 Tagen (pH = 5,2)
(d) Salzfr. Kontrolle	5,7	5,2	5,2	5,2	—

Key: a. mol/liter salt addition
b. pH value after
c. chain breakdown after
d. salt-free control
Std = hours
Tagen = days

When prodigiosum cultures, which have grown in chains in salt-containing media, are reinoculated on normal dextrose agar, then we once again get short rods. We therefore tried to find out whether it is possible to produce cultures which grow both on standard dextrose agar and in certain salt concentrations, in the same form. Here we first of all have two possibilities, both of which we investigated. (1) By means of the gradual adaptation to slowly increasing salt concentrations, we wanted to produce strains which would always retain the rod form. (2) Bacteria cultures were always retained in the form of threads on salt-containing nutrient media, through many generations, in the hope that they might lose the capability of once again forming little rods on standard agar through their adjustment to this medium.

Both experiments covered a period of 20 months. However they were utterly unsuccessful in the sense that we failed to produce strains which can produce the same form both in salt media and on standard agar.

Parallel to the inhibition of the growth and the partition, we also have a general drop in the vitality and connected with this we have a reduction in the pigment formation and an increase in slime production. With very few exceptions (KBr, KNO_3, $NaNO_3$), all of the thread and chain cultures produced by salt additions were either white or, at most, they were a pale pink; in analogy to the wide variety of transitional forms from cocci rods to the short chain, we get all of the intermediate shades from brick-red via pink all the way to white. A certain cell form here cannot be equated or associated with a special color tone; instead, each salt acts first of all on the shape and then on the color in a specific manner.

The slime formation increases along with the concentration of the salts added. Certainly, the slime envelope is not without significance for the subsequent breakdown and one can easily see that a considerable energy expenditure is required in order to overcome the resistance of the viscious slime layer during partition. The development of a slime layer perhaps also is responsible for the extraordinarily long drawn-out form of the long threads, such as we get them for instance when we add Cs_2SO_4.

Salt cultures are motionless without exception; the flagella apparatus is not damaged only when we add very small quantities of salt. The degree of inhibition depends on the chemical peculiarity of the salt used and we can set up an inhibition series for the flagella apparatus for the specific case of Bact. prodigiosum which will be related to Hofmeister's series; such an inhibition series for the flagella apparatus was found by Weinland for the ciliary epithelium movement in the throat mucosa of the frog:

Anions: $SO_4 <$ Tartrate $<$ Cl $< NO_3 <$ Br
increasingly damaging effect $\longrightarrow$

Cations: $K <$ Na, Rb $< NH_4 <$ Li $<$ Cs
increasingly damaging effect $\longrightarrow$

Summary

Addition of salts of the most varied cations and anions in Bacterium prodigiosum lead to the formation of normal individual cells or more or less long chains and threads. The development of the particular form depends on the position of the cation or the anion in the swelling series; the more strongly swelling the particular ion acts, the more strongly will the thread form appear via the chain form:

de-swelling ←——————————→swelling
cocci chains threads

Combinations of the ions, respectively, of their salts, either produce an additive effect or an antagonistic effect.

The addition of a salt has no effect if it is added later than 8 hours after inoculation of the culture. The cause is the then commencing acid formation, in accordance with the effect of the hydrogen ions which promotes the breakdown of the chains.

A certain carbon/nitrogen ratio is decisive for the length of the chain; the salt volume which will trigger the chain or thread formation however cannot be influenced by this ratio.

Pseudomonas fluorescens behaved in a similar manner and Bacterium coli behaved along these lines, although in a somewhat reduced manner. Bacillus mesentericus revealed corresponding reactions only in certain cases; however -- in contrast to the behavior of the other bacteria -- we did have a formation of threads when lithium salts were added.

Bacillus subtilis and one proactinomycete did not reveal any capability of being influenced here. Bacillus mycoides likewise generally did not react although it was possible, in this case, to achieve growth in individual cells through continuing over-inoculation of 3-week old cultures on nutrient media with a high dextrose content.

Experiments aimed at establishing partition-promoting or partition-inhibiting substances in bacteria cultures came out negative.

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